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Prone to fix: Resilience of the active nitrogen-fixing rice root microbiome

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Due to water consumption, many lowland rice areas in Asia are undergoing a transition that involves adoption of new management strategies, with crop rotations encompassing a non-flooded crop, including maize. Shifting from flooded to non-flooded cropping is likely to affect microbial nitrogen cycling. For analysis of the root-associated microbiome of rice and maize in response to flooding or nitrogen fertilizer, we combine methods of microbial ecology (Next-Generation sequencing of amplicons), and a reductionist approach with pure cultures of the endophytic diazotroph Azoarus sp.. Field plots of the ICON project (Introducing non-flooded crops in rice-dominated landscapes: Impact on Carbon, nitrogen and water budgets) at the International Rice Research Institute in the Philippines were analyzed. Root-associated activity of nitrogenase gene expression was assessed by quantitative RT-PCR of nifH. For rice, expression levels were surprisingly stable, in response to non-flooded versus flooded conditions, or in response to conventional nitrogen fertilizer applications versus lack of N-fertilizer. In contrast, the active diazotrophic population of maize roots was not resistant to N-fertilization, nifH expression strongly decreased. Concordant changes in the diazotrophic resident or active communities were detected by nifH amplicon sequence analysis, based on bacterial DNA or mRNA, respectively.
For high-resolution analyses of the endobiome in gnotobiotic culture, we developed a dual fluorescence reporter system for Azoarcus sp. BH72 which allows to quantify and visualize epi- and endophytic gene expression by concfocal microscopy (CLSM). This allowed us to demonstrate sites of active nitrogen fixation (gene expression) in association with rice roots. We confirmed that at low nitrogen fertilizer levels, endophytic nifH gene expression persisted in rice roots, while it was repressed in maize roots. This supports our observation of remarkable stability of nitrogen fixation in association with rice roots.